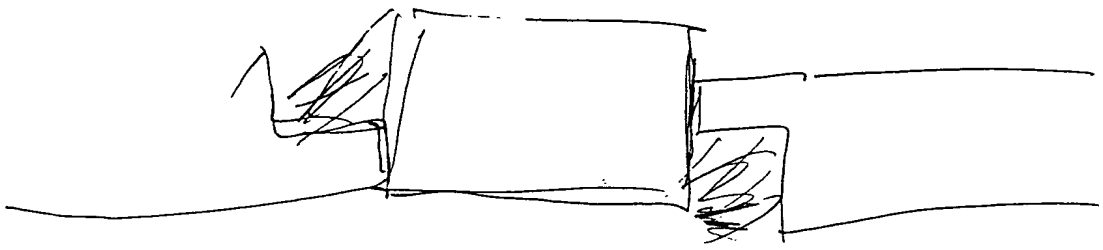
Reverse Complement DNA Sequence 09•YD - 19.Seq(1,263)

...

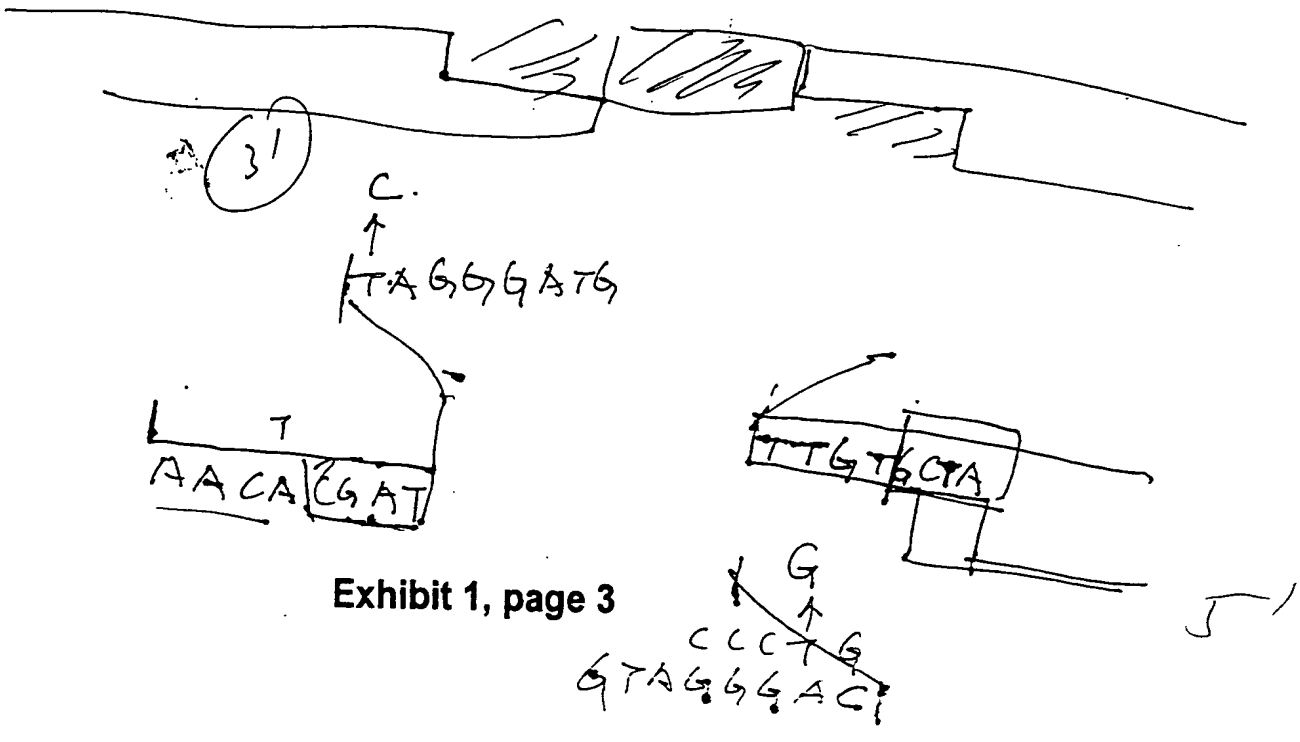
```
          10        20        30        40        50        60
          |         |         |         |         |         |
TTTTNGTTTT TTACCTCGGG TTNGAAATCG ATCGGGATAA AACTAACAAA ATCGGTTATA 60
CGATAACGGT CGGTACGGGA TTTTCCCATC CTACTTTCAT CCCGGGCTAC AAGGCTTCCC 120
AAGCTCACTC GGGAGCAACA GGATCTATTG TGGTGGAGTC GGGTCGGGTC AGGTTATGAT 180
CGACCCGGTT ATTTCTCCAT GGGTTTTGTT GAGACTTCCT TCC 223
```

3' - cDNA 15' D3

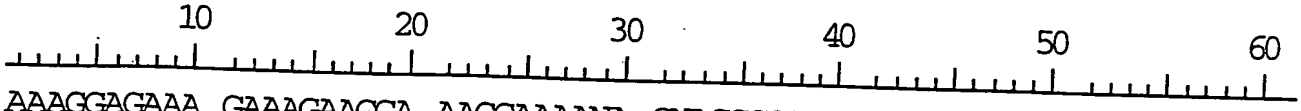
```
          10        20        30        40        50        60
          |         |         |         |         |         |
TTTTCGTTTT TTACCTCGGG TTCNAAATCG ATCGGGATAA AACTAACANA ATCGGTTATA 60
CGATAACGGT CGGTACGGGA TTTTCCCATC CTACTTTCAT CCCGGGCTAC AAGGCTTCCC 120
AAGCTCACTC GGGAGCAACA GGATCTATTG TGGTGGAGTC GGGTCGGGTC AGGTTATGAT 180
CGACCCGGTT ATTTCTCCAT GGGGTTTTGT TGAGACCTCC TCCACTACTC ATGAGCTCTC 240
TTCANT 246
```



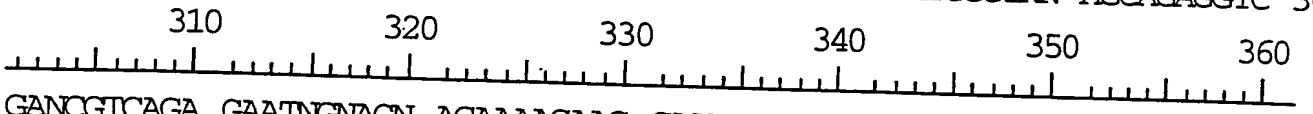
```
          10        20        30        40        50        60
GTAGCATCGA TCTCTAACAA CGCTACCCGT TTACCCGTAC CGGTAGACCC GGGTGTTGTG 60
CTACAGGGAT GAAAACGGTC GGTAACGGTC GGTAAAATAC CTCTACCGTT TTCATTTTCA 120
TATTTAACTT GCGGGACGGA AACNAAAACG GGATATACCG GTAACNAAAA CGAACGGGAT 180
AAATACGGTA ATCGAGTGnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnTT TTCGTTTTTT 240
ACCTCGGGTT CNAAATCGAT CGGGATAAAA CTAACANAAT CGGTTATACG ATAACGGTCG 300
          310       320       330       340       350       360
GACGGGATT TTCCCATCCT ACTTTCATCC CGGGCTACAA GGCTTCCCAA GCTCACTCGG 360
GAGCAACAGG ATCTATTGTG GTGGAGTCGG GTCGGGTCAG GTTATGATCG ACCCGGTTAT 420
TTCTCCATGG GGTTTTGTTG AGACCTCCTC CACTACTCAT GAGCTCTCTT CANT 474
```



Reverse Complement DNA Sequence 22•YWC - 282-33-2.Seq(1,699)



AAAGGAGAAA GAAAGAAGGA AGGAAAANA GNAGGGGGGA AAGAAANGGN NANGAGNNAA 60
GAAGANAGGA AGTGAAGGGG AGGGGAAGNG GAGAAAGGGG GAANGNTTAA TNTTANGTNA 120
GNGGTNNNAA ATTCTGTGAG AAACCCNGGT GATTTTATGA GGGACACCNG TGTTATNGTC 180
AATAGANNAN GAGAGATNCG GACAGAGACA CTGAAGAGTN NTNGTNGGAA ACCAGGATCG 240
^AGACANGTC AACAGAGACN GNNGNAACCA ACGTTGAGAG GAATGGGTNN AGCAGAGGTC 300



GANCGTCAGA GAATNGNAGN AGAAAAGAAG CAANTCACCN CCNCCACAGT CGGAGACACG 360
TCATCAGTAN CNTNGATATC TAACCACGTT ACCCGTTNAC CCGTACCGGT AGACCCGGGT 420
GTTGTGCTAC AGGGATGAAA ACGNTCTGGT ANCGGTNGGT TATATACATT TAACCTTGTT 480
TNGTNTTTNA AAGTNAACTN TGAGNGNCGT GAA 513

Large scale production of Flexible Seq.

- use the original 1:50 sediluted of 2nd round T-PCR product. ~~78~~ 78, 81, 33 and redilut of 36/2:50

-	78 (SGT~~736~~ 782):	Ds5-3/AD3	12
-	36 (SGT736):	Ds3-3/AD3	12
-	81 (SGT736):	Ds5-3/AD3	24
-	33 (SGT282):	Ds3-3/AD3	24

Cocktail:

	A: for 33 and 36: 37 sets Ds3-3/AD3	B: for 78 and 81: 37 set Ds5-3/AD3
~~x Buffer~~	74	74
2gel2	59.2	59.2
H2O	432.9	432.9
dNTP	6.0	6.0
Ds primer	14.8	14.8
AD primer	74.0	74.0
poly.	5.5	5.5
	666.4	666.4

33: 444.2 µl
WA: ~~24 µl~~

81: 444.2 µl
DNA: ~~24~~ 24.6 µl

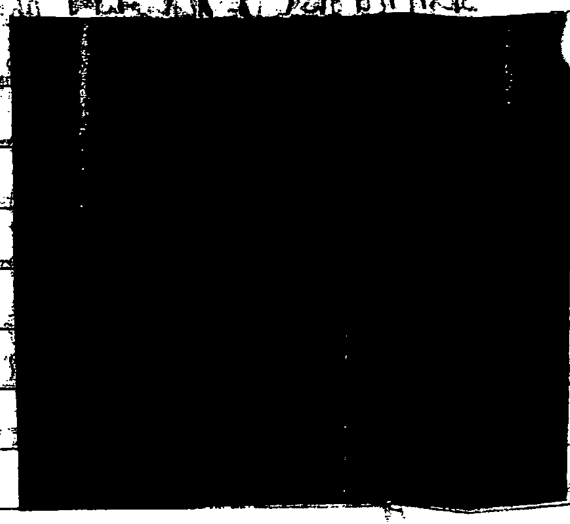
36: 222.1 µl

78: 222.1 µl

Large scale production of 282/736 Handing 3/5

Test running: 2% agarose gel. TAG

1kb ladder, 2nd ΘP, 3rd ΘP, run 37, 2nd ΘP, 3rd ΘP, run 37, run 36, run 78 } ~~Good~~ Good



- OK.
- purify the gel rest of 33. DST-3/AD3
  81: DST-3/AD3

recovered: 18ul × 21 = 378 ul
added 6X loading buf. 73.6 } = 451.6ul.

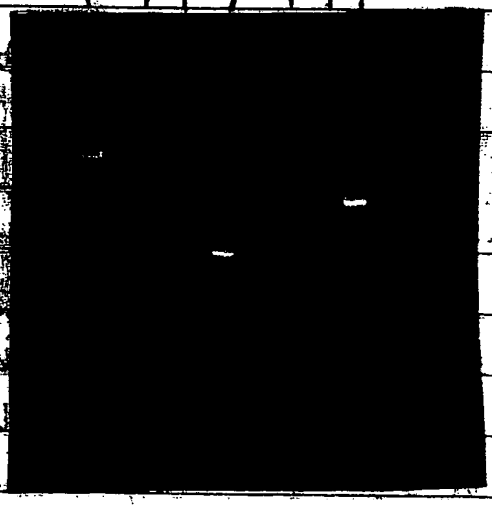
- run 1% gel.

- cut out the bands. #33: 300mg × 3
  #81: 300mg × 5 (could be contaminated by #33 throw by mistake using the blade for #33
  Anyway, should not

- store the gel block at 4°C
  O/N ?

1. Qiaquick - gel column - purify the fragment.

3 column / each sample. - multiple loading
- twice wash.
- elute in 50μl x 3 = 150 μl H_2O
- run ~~10%~~ 10 μl in 2% agarose gel.



smaller: SGT736 5'/AD
Larger SGT282 - 3'/AD

stored: Box #IL-1 -20°C
- SGT736 5'/AD3: #81-3 (Larger)
- SGT282 3'/AD3: #33-3 (Largerscal)

- run the second-round - Large-scale - production - of #33, #81 and #78.
Larger

- see the result on page 54.

- the result of the 2nd. 3nd round PCR products of Large scale PCR

#33: ~1Kb

#f1: 0.5Kb

#78

L: Large : 1.5 Kb

M: Medium : 0.5Kb

S: Small. : 0.3 Kb

- 1 2 3 - 4 5 6 - 7 8 -

#33 #f1 #78

3.6. 5ul out of 50 ul Larger scale (see page 50-53)

See page 46 for the background.

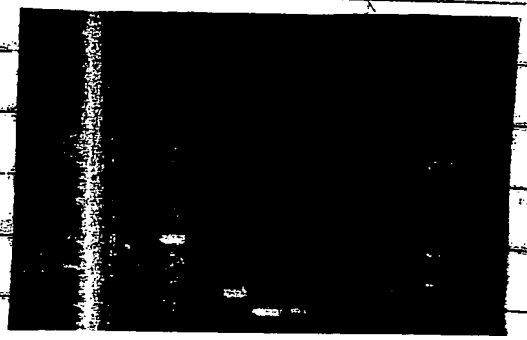
Notes:

- the Q-purified bands (3) run slower (Larger) than its original PCR product (2). why? Cut out the #1~3 bands purified by Q-f column to check it out (see the photo below for ~~ex~~ the bands

- #78 positive is a biggest good news: SGT78: 5' Flanking 50. the Flanking sequences from both ends have been already out

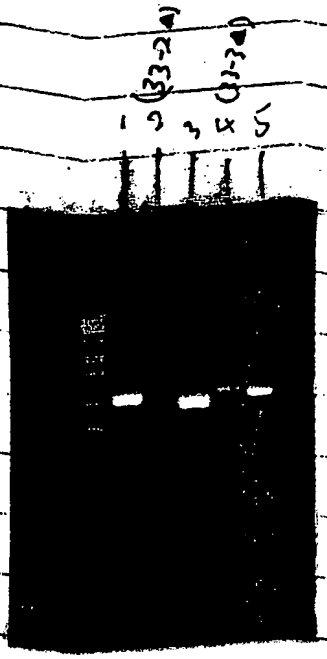
3'-end DS3/AD3: ~~Cut~~ 3 bands: L 1.5Kb. M 0.5Kb. S 0.3Kb.

5'-end DS5/AD3. single band: ~500pb



Q-Q column purified the cut-bands of #33-2, #33-3 and #78-3.

- #78-3 (SGT282.5'/AD3) was passed to YCK for cDNA screening
- Run the #33-2, #33-3 compared with the PCR original's



SGT282 DS5/AD3

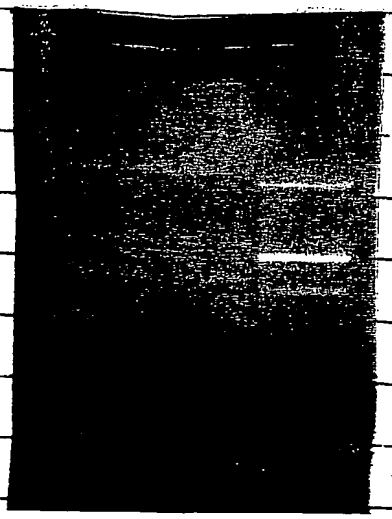
1. original 2° PCR product. 10 µl
2. Q-purified 2° PCR product. 10µl out of 50µl
3. original 3° PCR product 10 µl
4. Q-purified 3° PCR Product 10µl
5. 5 µl Q-purified 3° round Larger scale production.

Conclusion OK.

stored: Box#FL-1 -20°C

SGT282 3'-FL. 2° round (2): 33-2

SGT282 3'-FL. 3° round (4): 33-3



P-Lab: meeting: on duty. tuskin

SGT 282

- Run & purified the SGT282-5' Flak
- Cut out the 3 different size bands
- stored at 4°C

Q purification elute in 30µl

282-4AT3CRxx4AmfCR Map (1 > 1224)Site and Sequence
Enzymes : All 206 enzymes (No Filter)
Settings: Linear, Certain Sites Only, Standard Genetic Code

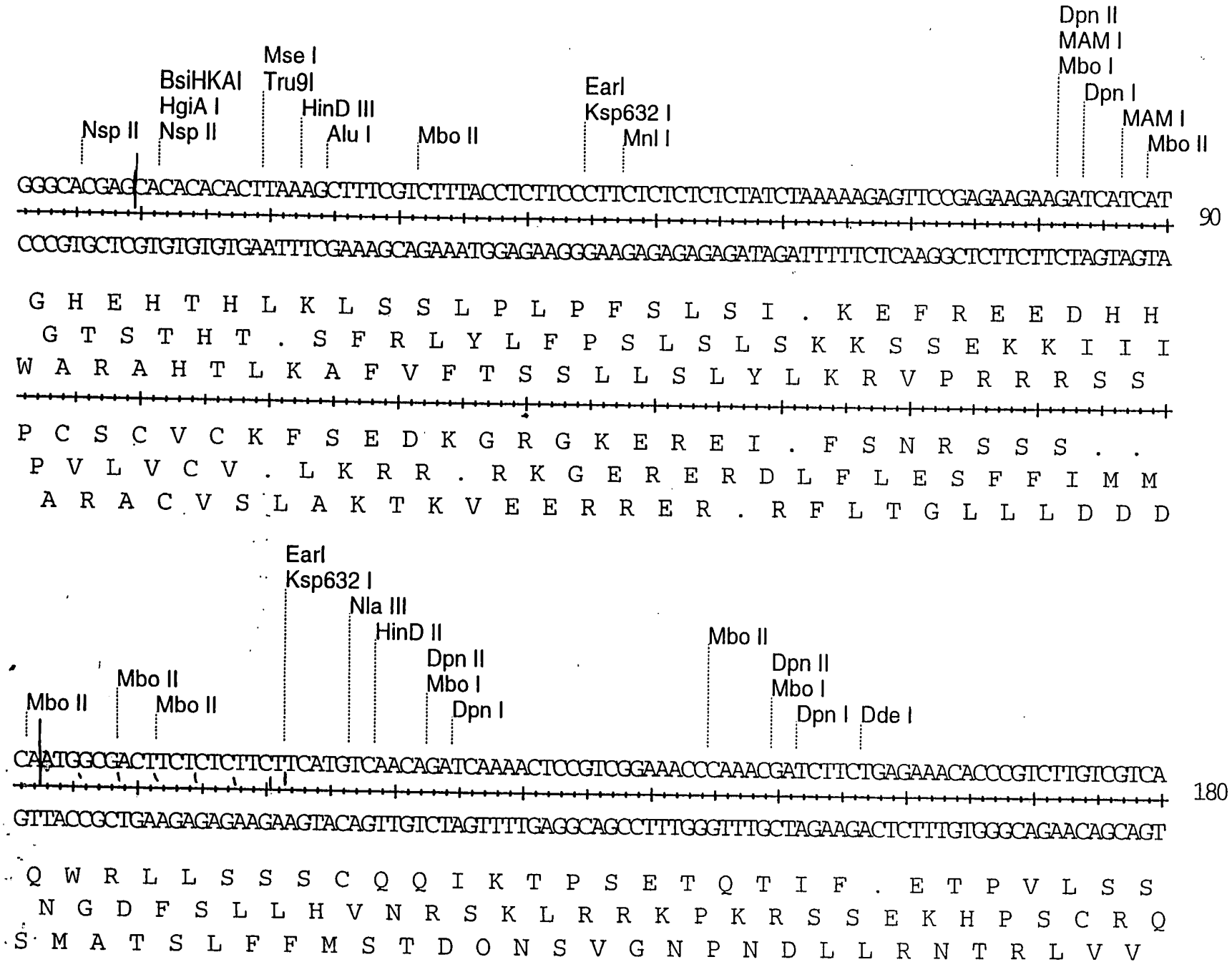


Exhibit 3, page 1

. H R S R E E E H . C I L V G D S V W V I K Q S V G T K D D
L P S K E R R . T L L D F S R R F G L R D E S F C G D Q R .
I A V E R K K M D V S . F E T P F G F S R R L F V R R T T L

Bin I
Dpn II
Mbo I
Xho II
Dpn I
Bca77I
BsaWI
BspM II
Alw26 I
Hpa II
EarI
Ksp632 I
HinF I
Ple I
Mbo II
EcoR II
Eco57 I
Apy I
ScrF I
Bin I
Dpn II
Mbo I
Dpn I
Taq I
Bbv II
EcoP I
Bbs I
Bpu10 I
Bsc91I
Mbo II
MnlI
Mae II
Psp1406I
Alu I
Hpa II
BsmA I

ATAGCTCCGGCGAGATCCGGACAGAGACACTGAAGAGTCGTGGTCGGAAACCAGGATCGAAGACAGGTCTGCAAAAACAGAAGAAACCAA 270
TATCGAGGCCGCTCTAGGCCTGTCTCTGTGACTTCTCAGCACCAGCCTTTGGTCCTAGCTTCTGTCCAGACGTTTTTGTCTTCTTTGGTT

I A P A R S G Q R H . R V V V G N Q D R R Q V C K N R R N Q
. L R R D P D R D T E E S W S E T R I E D R S A K T E E T N
N S S G E I R T E T L K S R G R K P G S K T G L Q K Q K K P

L A G A L D P C L C Q L T T T P F W S R L C T Q L F L L F W
Y S R R S G S L S V S S D H D S V L I S S L D A F V S S V L
L E P S I R V S V S F L R P R F G P D F V P R C F C F F G V

Exhibit 3, page 2

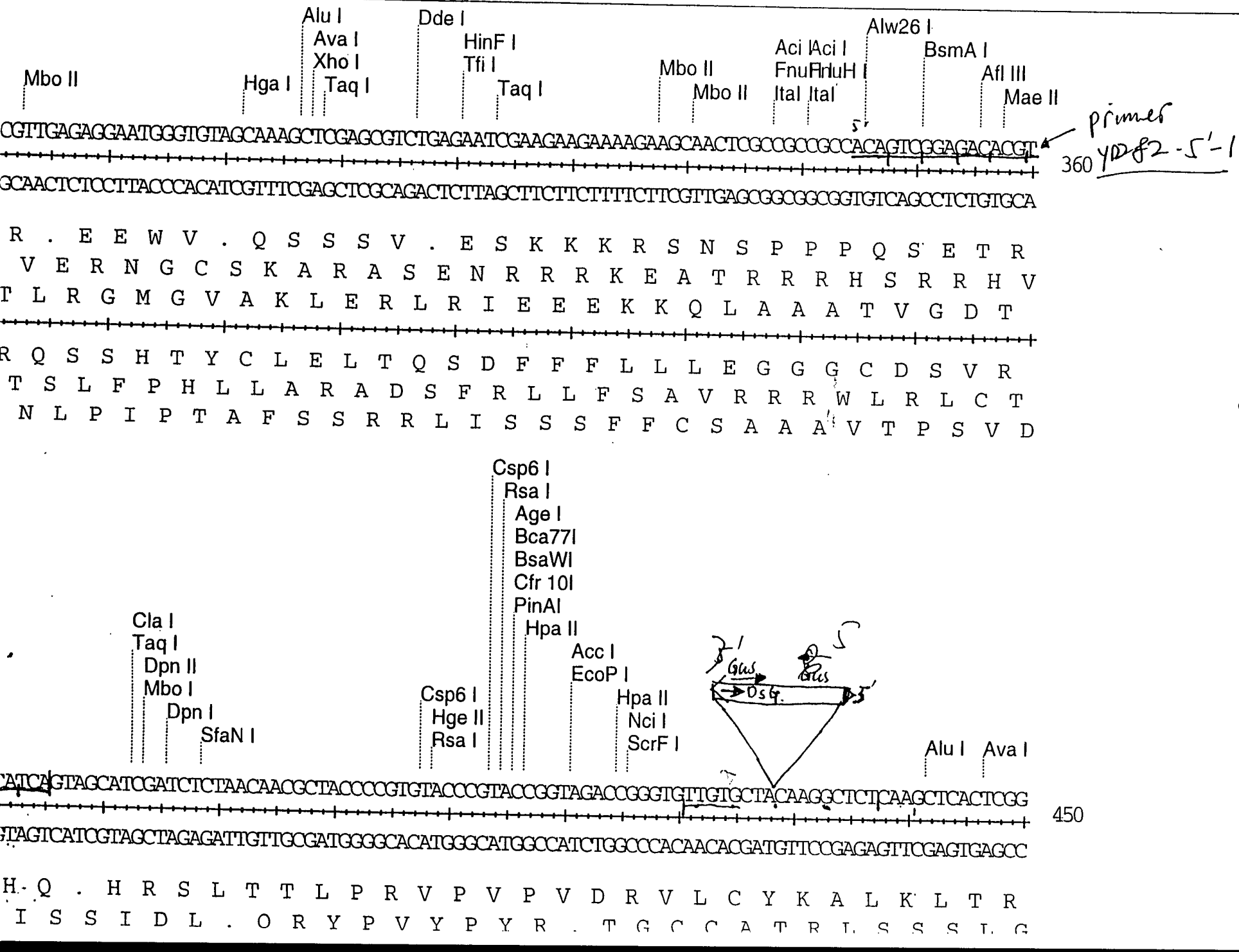


Exhibit 3, page 3

282-4AT3CRxx4AmfCR Map (1 > 1224)Site and Sequence

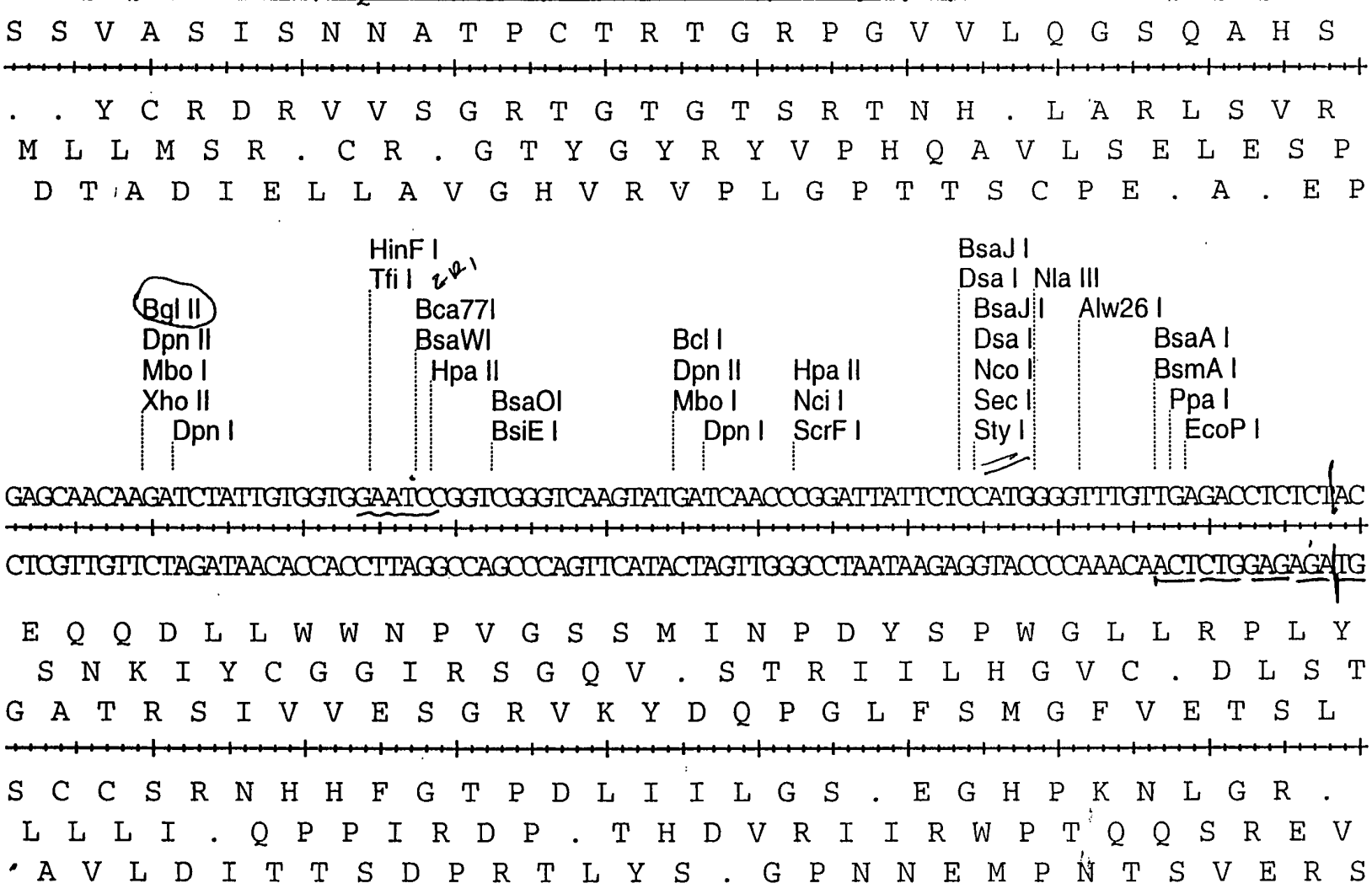


Exhibit 3, page 4

282-4AT3CRxx4AmfCR Map (1 > 1224)Site and Sequence

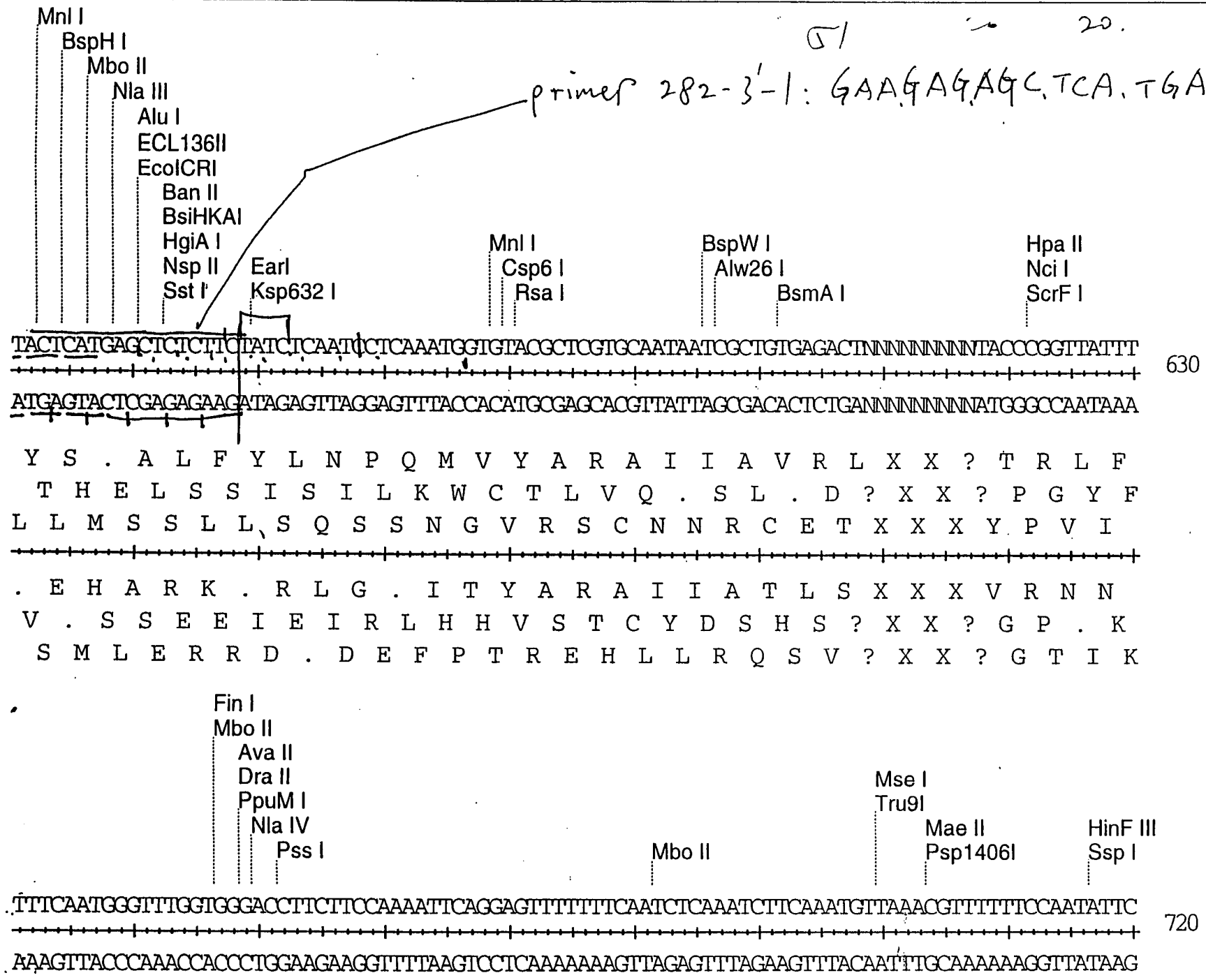


Exhibit 3, page 5

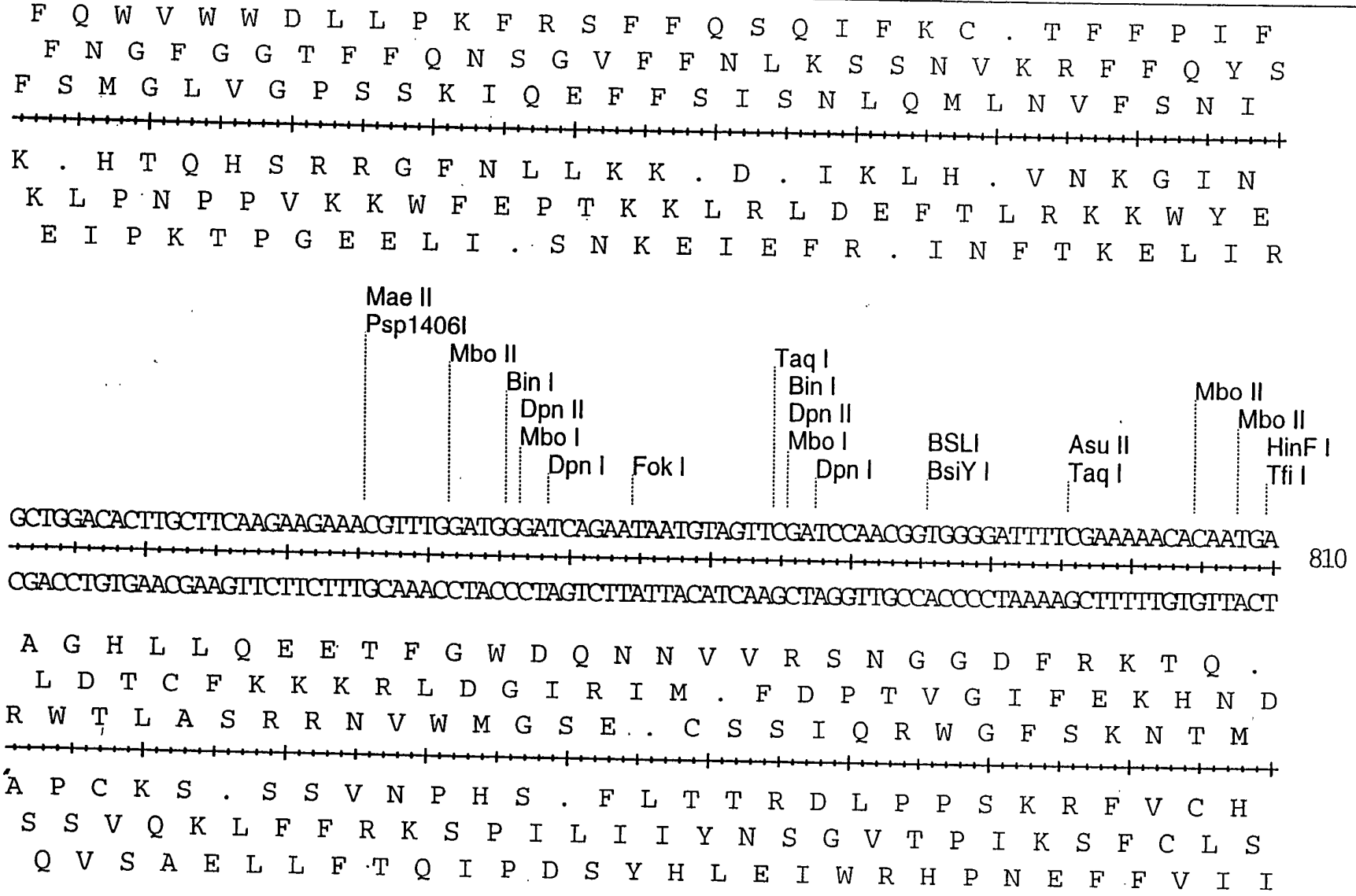


Exhibit 3, page 6

282-4AT3CRxx4AmfCR Map (1 > 1224)Site and Sequence

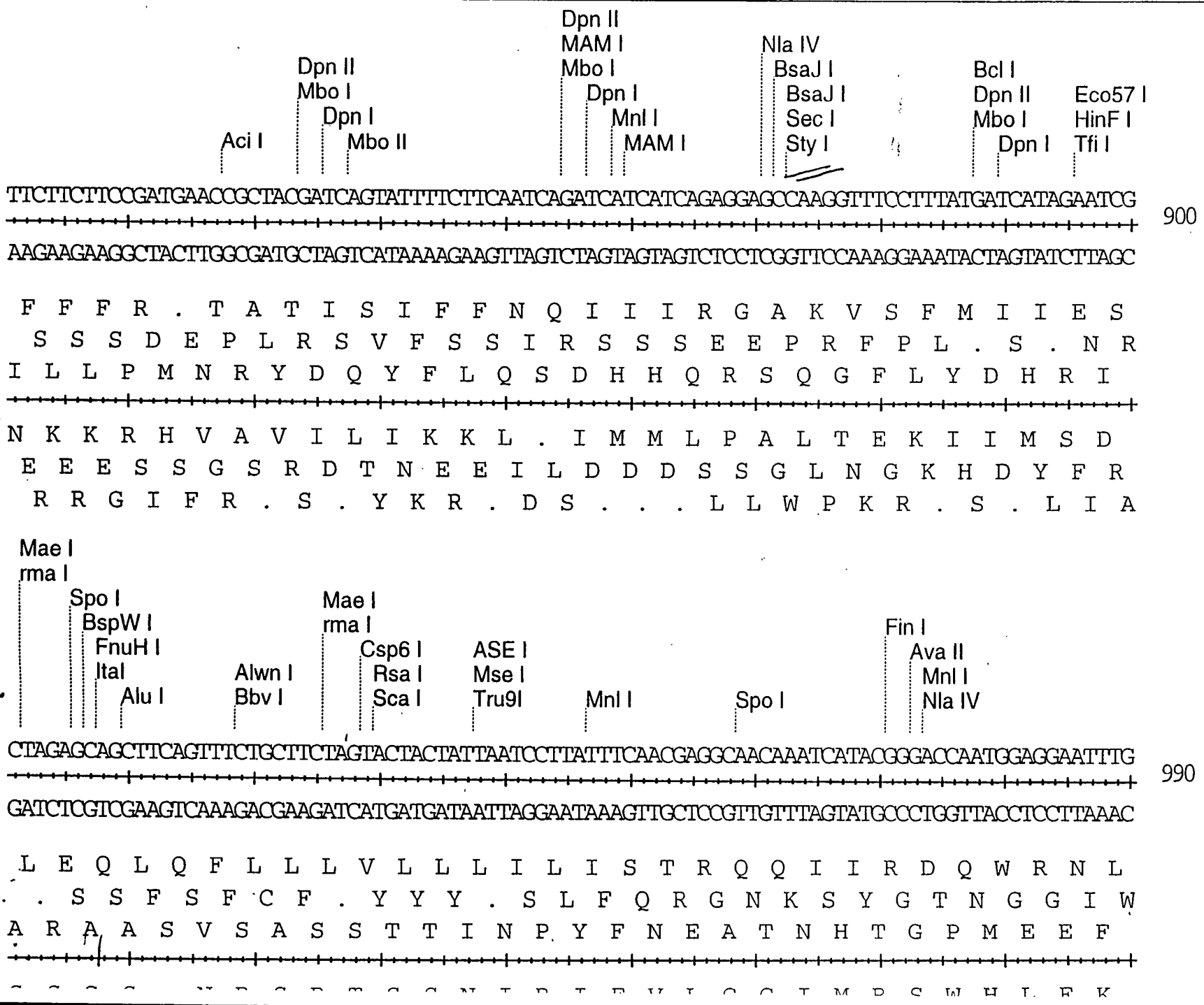


Exhibit 3, page 7

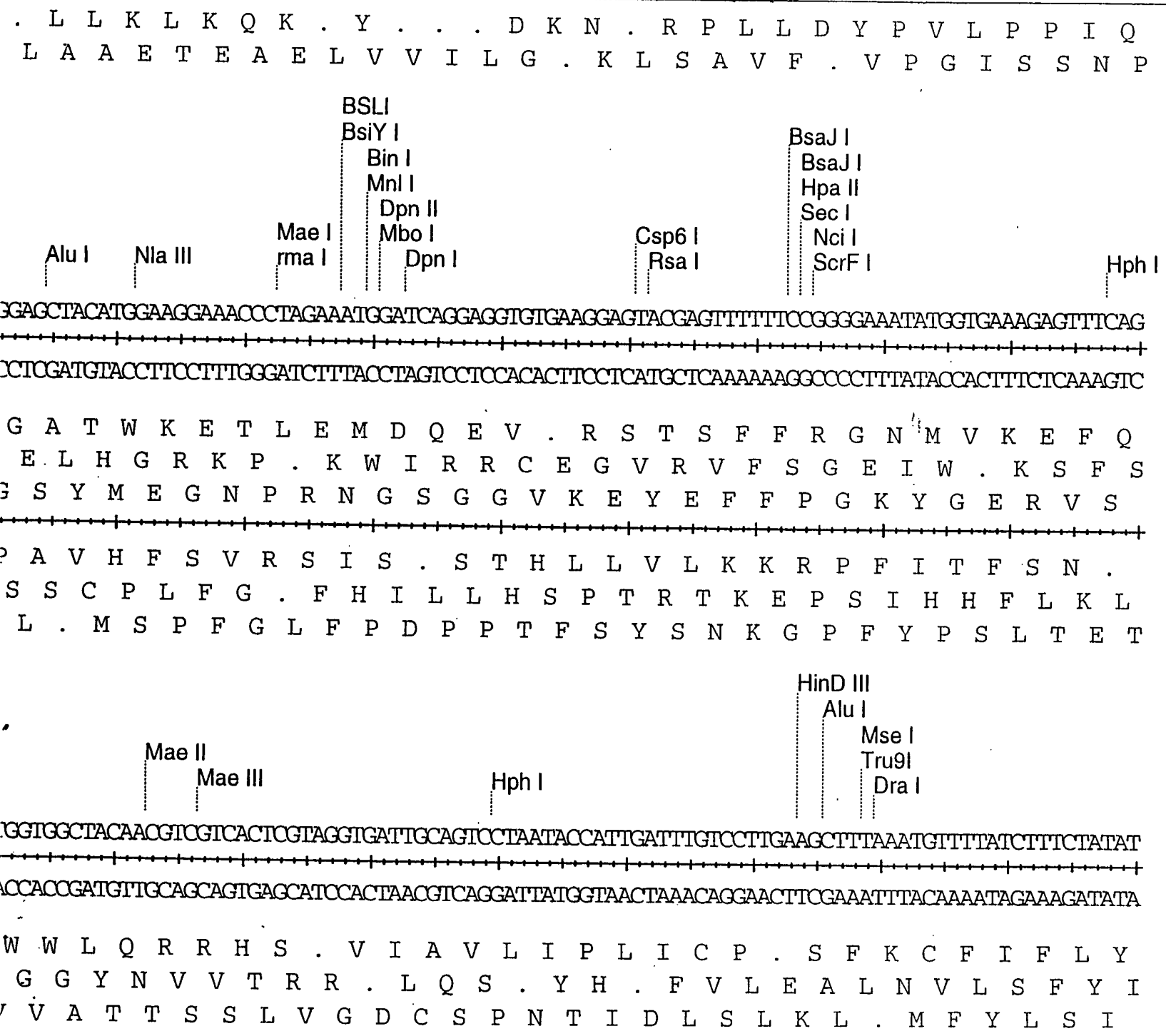


Exhibit 3, page 8

H H S C R R . E Y T I A T R I G N I Q G Q L K L H K I K R Y
P P . L T T V R L H N C D . Y W Q N T R S A K F T K D K . I
T A V V D D S T P S Q L G L V M S K D K F S . I N . R E I N

BsmA I
Mse I
Tru9I
Mse I
Tru9I
Dra I
Esp3 I
Alw26 I
Mse I
Tru9I
Dra I

IGATTTAAACAAAATCGTCTCTTTAAAGAAAAAACATTTTAAGTAGATGAAAGT
ACTAAATTTGTTTTAGCAGAGAAATTTCTTTTTTGTAAAATTCATCTACTTTCA 1224

. F K Q N R L F K E K T F . V D E S
D L N K I V S L K K K H F K . M K V
L I . T K S S L . R K N I L S R . K

Q N L C F R R K L S F V N . T S S L
S K F L I T E K F F F C K L Y I F T
I . V F D D R . L F F M K L L H F H

Exhibit 3, page 9

Titering Library.

1 colony ·1RF or 100µl

↓ ↓

5ml LB + 0.2% maltose + 10µM MgSO4 10ml LB+—

↓ ↓

O/n 37°C — OD=1.63 37°C 2-3hr

↓ ↓

0.5ml — in 5ml LB +0.2% maltose +10mM MgSO4

↓

1.5 hr. OD_{600} = 0.598

↓

spin down 4000 rpm 4min

↓

10mM MgSO4

↓

600µl ← 14µl ← 1000µl sam ← µl phage Library

14µl good

↓

15°C 37°C

↓

Plating

↓

37°C 8hr.

Sequence		primer			
1	SGT26	80-2	D2-2		
2	SGT26	80-3	D2-3		
3	SGT26	82-	D33-2		
4	SGT82	82	D3 3-3		
5	M3-5-1		T3 (10pm)		
6	M3-5-1		T3		
7	M7-1-2		T3		
8	M7-1-2		T3		

PCR #1 = 60 + 1

60, 95°C

50°C

Exhibit 4, page 2

Screening for SGT282 cDNA. Hoover library

1° — 14 plate (≈ 700,000 phages) were screened with SGT282 probe (10 μl from each PCR amplification including both 3' and 5' flanking sequence) (1 μl library — 1000 μl → 35 μl/plate

↓

Washed 2×SSC, 1% SDS for 65°C 2hr.

↓

exposed with intensifying screen. 24 hrs

↓

5 positives → 500 μl SM + chloroform 4°C

SGT282-1, 282-2, 282-3, 282-4, 282-5

2° — positives were picked up and store in 500 μl SM + chloroform o/n.

dilution 1 μl — 100 μl SM

plating: 10 μl, 20 μl, 50 μl / plate respectively

282 No.: 1 2 3 (282 1-1, 1-2, 1-3)

↓

Second screening × 65°C hybridization, ×2SSC, RT

↓

No.: 282, 1-1-1, 1-2-1

2-1-1 - -

3-1-1, -

4-1-1 -

5-1-1, - 5-1-1-2 ...

Third screening. ×65°C, H, ×2SS Wash RT

No. 282 3-1-1-1, ① ② ...

1/3 (50-100) were positive 4-2-4-2, ① ① — from the same plates

5-1-1-1, ① ② -

↓

only 3, 4, 5 were positive at the third screening

No. 1, and 2 were lost

In Vivo excision

No. 3-1-1-1, ② 3-1-1-2 ① 3-1-1-2 ② **Exhibit 4, page 3**

4-2-4-2 ① ②, 4-2-4-1 ① 4-2-4-1 ②

84

single phage ~~colony~~ picked up and in LB/O overnight
single colony was picked up and cultured in 20ml C6
+ 50μg/ml Amp 37°C. 10hr.
DNA were isolated using QIAgen-tip 100.
dissolved in 20 50 μl water. too much salt
Then diluted into 300μl and ethanol precipitate.
Spin in speed Vac. and dissolve into 100μl water

EcoR I — Xho I cut.

5μl DNA
1μl EcoR I
1μl Xho I
3μl 10x UB buffer (Stratagene)
10μl H2O
20μl
37°C o/n.

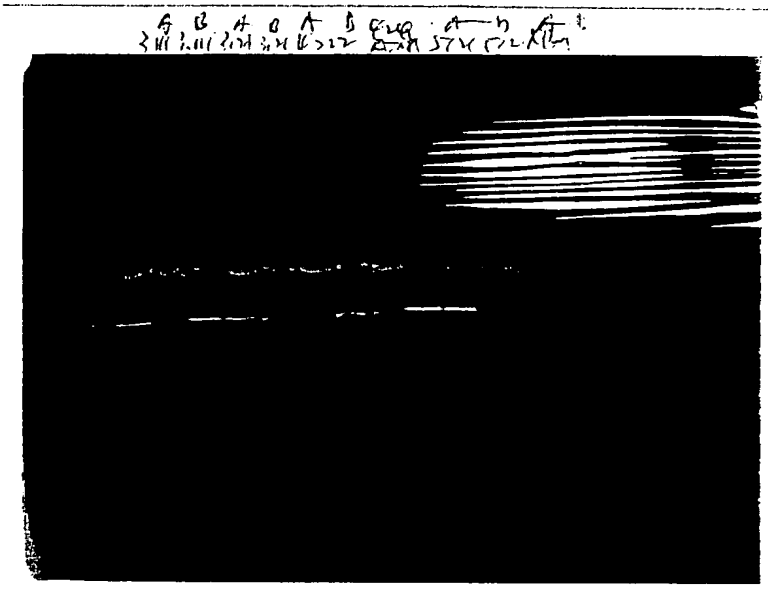


Exhibit 4, page 4

Sequencing 282 candidate

No. of tube	Sample	primer
~~1~~	1	282-3111A	T3 > ywc282-3T3/T7
~~2~~	2	"	T7
~~3~~ ~~4~~	3	282-4-2-4-2A	T3 ywc282-4T3
	4	"	T7 T7
	5	282-5121A	T3 ywc282-5T3
	6	"	T7 ywc282-5T7
	7	~~28~~ M3-1A	T3 ywc3-1AT3
	8		T7 ywc3-1AT7
	9	282-5121B	T3 ywc282-5BT3
	10	"	T7 YWC282-5BT7

8 µl Terminator mix
~~20~~ µl primers (20µl total)
10µl DNA
~~total~~ 40µl

T7 promoter do not work.

New Sequencing is carried out as follows

PCR tube	sample	primer	mark for label
1	282-3111A	T3	282-3AT3
2	282-3111A	T7 (from Xue Yuan)	282-3AT7
3	282-3111A	M13 Forward	282-3AMF
4	282-3111A	M13 Reverse	282-3AMR
5	282-4242A	T3	282-4AT3
6	"	T7	282-4AT7
7	"	M13FP	282-4AMF
8	"	M13 RP	282-4AMR
9	282-4222A	T3	282-4BT3
10	"	T7	282-4BT7
11	"	M13 FP	282-4BMF
12	"	M13 RP	282-4BMR